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The administration of exogenous prostaglandin may improve ovulation in pacu (*Piaractus mesopotamicus*)

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Abstract

Based on the reports of unsuccessful ovulation in pacu (*Piaractus mesopotamicus*) by fish farmers and researchers undertaking artificial reproduction programs, we evaluated the use of prostaglandin F (PGF) to improve pacu ovulation. This study was conducted during two spawning seasons (2009/2010 and 2010/2011) with two samplings in the first season and one sampling in the second season. A total of 45 females was sampled in this study. The control group was injected with carp pituitary extract (crude extract, 6 mg/kg), and the treatment group received PGF (2 mL per fish in the 2009/2010 season and 5 mL per fish in the 2010/2011 season) in addition to the crude extract. In both seasons, 100% (N = 4, 2009/2010 first sampling; N = 5, 2009/2010 second sampling; and N = 3, 2010/2011) of the PGF-treated fish spawned. In contrast, 53.0% (N = 9) and 83.3% (N = 10) of the control fish spawned in the first and second samplings of the 2009/2010 season, respectively, and only 25.0% (N = 1) spawned in the 2010/2011 season. Fecundity, fertility, and hatching rates did not differ ($P > 0.05$) between the treated and control fish. Based on oocyte volume frequency analysis, ovaries of the control fish had more ($P < 0.05$) vitellogenic oocytes with germinal vesicle breakdown that remained unovulated after spawning, whereas more ($P < 0.05$) of previtellogenic oocytes were present in the ovaries of the PGF-treated fish. In conclusion, administration of exogenous prostaglandin may improve the outcome of hormonally induced spawning in tropical migratory fish.

Keywords: Pacu; Prostaglandins; Ovulation; Ovarian follicles

1. Introduction

Pacu (*Piaractus mesopotamicus*) are large tropical fish classified as herbivorous/frugivorous characiform used for aquaculture. The species is cultivated in different regions of Brazil [1,2], mostly in the southeastern region [3]. The production of pacu has increased from 12 397 tons in 2007, and 15 189 and 18 171 tons in 2008 and 2009, respectively [4]. Nevertheless, *P. mesopotamicus* is a total spawner, and hormonal stimulation is required for the species to spawn in captivity. Reproduction occurs between October and March, when the highest water temperatures occur (28 °C to 30 °C) and pluviometric concentrations are typically recorded [3].

However, hormonal treatment can be unsuccessful, and unpredictable ovulation becomes a common limitation because of the need for hormonal induction [5]; this is one of the main limitations in pacu fingerling production. In another tropical migratory fish (*Brycon amazonicus*), our group recently reported that this dysfunction resulted from the retention of oocytes that

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germinal vesicles had broken down (GVBD) within unbroken follicles [6].

Final maturation is a prerequisite for ovulation, which involves resumption of meiosis. This phase normally occurs during the first few hours after the initial hormonal dose, and it is characterized by the hydration of oocytes and the initiation of GVBD [7]. When final maturation is complete, ovulation begins and oocytes are released from the ovarian follicles. The process of oocyte release consists of the rupturing of the connections between the microvilli of the granule cells and the oocytes, narrowing and disruption of the follicular wall and contraction of the smooth muscles of the follicular wall [8]. Moreover, these processes are regulated by hormones. Progesterone 17α, 20β-dihydroxy-4-pregnen-3-one (DHP) is one of the main maturation-inducing hormones [7]; in addition, its precursors and metabolites as well as many other substances, including prostaglandins, also have important roles in final maturation and ovulation. Prostaglandins play an especially important role; they are eicosanoids derived from arachidonic acid and have been identified promoting ovulation in several teleosts [9–12]. Although a limited number of these substances have been exploited for induced reproduction procedures, prostaglandins are strongly associated with the process of ovulation in fish [13,14].

Goetz and Cetta [11] reported significant increases in plasma and ovarian prostaglandin F (PGF) concentrations in naturally ovulating, compared with nonovulating, *Salvelinus fontinalis*. In *Perca flavescens*, DHP was reported as a release inducer of prostaglandin E (PGE) and PGF within in vitro mature follicles [15]. In addition, Bradley and Goetz [16] reported a dose-dependent relationship between ovulation and the concentrations of PGF and PGE. Based on in vitro analysis of *Anguilla japonica*, DHP induced ovulation through the synthesis of endogenous prostaglandin in the follicular layers [17]. In light of these observations, the aims of the present study were to evaluate the possibility of improving the rate of pacu females that spawn after hormonal induction and also to determine whether exogenous PGF could improve spawning success.

2. Materials and methods

2.1. Animals and treatments

During two consecutive spawning seasons (2009/2010 and 2010/2011), 45 *P. mesopotamicus* females were sampled. Males and females (at a sex ratio of 1:1) were maintained at the Duke Energy Facilities, Brazil, located in Salto Grande, São Paulo, Brazil (22° 53′34″ S and 49° 59′08″ W). Fish were distributed in 200 m^3 earthen ponds with a stocking density of 0.25 fish per m^3 and with a constant water flow between 15 and 20 L per min. Females were randomly selected for the induced breeding experiments; the mean weight of the females was 1.8 ± 2.2 kg, and the mean length was 44.47 ± 1.44 cm.

Broodstock fish were transported to the lab for acclimatization in 500 L tanks before spawning. Four fish were held in each tank, and males and females were kept in separate tanks. The control and PGF-treated groups both received two doses of carp pituitary crude extract (0.6 and 5.4 mg/kg with a 24-h interval between the doses). The PGF-treated group also received a dose of synthetic PGF (Ciosin® containing 0.25 mg/mL cloprostenol, Schering-Plough Saúde Animal Ind. Com. Ltda. Campinas, SP, BR) at the time of the second crude extract dose. Experimental protocols were submitted to, and approved by, the Animal Ethics and Welfare Committee (Comite de Ética e Bem-Estar Animal) of The Faculdade de Ciências Agrárias e Veterinárias, University of Sao Paulo State, Jaboticabal, SP, Brazil (Protocol: 013162-11).

In the 2009/2010 season, two spawn samplings were performed; the first sampling was on 1/28/2010 (4 females treated [T1] and 17 females, control [C1]), and the second sampling was on 2/12/2010 (5 females treated [T2] and 12 females, control [C2]). Each fish in the treatment group received 2 mL PGF. In the 2010/2011 season, a single spawn sampling was performed (3 females treated [T3] and 4 females, control [C3]), and the fish in the treatment group received 5 mL prostaglandin (Table 1). The dose was increased from 2 to 5 mL because in the first breeding season, even among treated females, those that spawned early (approximately 10 h after the second dose) had greater fluidity in the release of eggs, whereas those that spawned later (more than 10 h after the second dose) were more difficult to strip (data not shown). Therefore, it was theorized that 5 mL would induce females to spawn early.

Table 1
Values of total weight (kg), length (cm), and gonadosomatic index (GSI) mean ± SEM of *P. mesopotamicus* control (C) and prostaglandin-treated (T) groups, sampled in the spawning season 2009/2010 (January [1] and February [2]) and spawning season 2010/2011 (February [3]).

	Variables			N
	Weight (kg)	Length (cm)	GSI (%)	
C1	1.95 ± 0.2	44.29 ± 1.2	18.92 ± 4.9	17
T1	1.94 ± 0.1	45.20 ± 0.3	16.71 ± 3.2	4
C2	2.00 ± 0.1	45.78 ± 0.6	18.33 ± 4.6	12
T2	1.80 ± 0.2	44.64 ± 1.7	23.17 ± 4.3	5
C3	1.40 ± 0.0	41.30 ± 0.0	17.89 ± 0.0	4
T3	1.50 ± 0.4	42.47 ± 3.4	14.99 ± 4.6	3

2.2. Reproductive performance

In all samples, the spawning rate (the percentage of spawning females) was determined using the following formula: the total number of spawned females/the total number of females × 100. The total number of oocytes released from each female was estimated; after spawning, the total weight of oocytes released by each female was recorded. Then, a gram of spawn was used to count the total number of oocytes per gram and made into tetraplicates. The total number of oocytes per gram was multiplied by the total weight of spawn; then, the total number of oocytes released was estimated.

After weighing, the oocytes of each female were fertilized using a pool of semen from males of the same broodstock, with at least four males used per sample. To avoid the effects of factors unrelated to the influence of the females during the artificial breeding process, the same pool of semen was used for the PGF-treated and control groups. Approximately 0.5 mL of semen was used to fertilize 50 g of oocytes. The sperm concentration in this species ranges between approximately 14 to 37×10^9 cells per mL [18]. The average number of oocytes present in a gram of spawn is approximately 1200 [19]. Therefore, the sperm:egg ratio used in this study was estimated at between 11.6 and 30.8×10^4 sperm per egg. After fertilization, eggs from each female were distributed into four 7-L incubators (four replicates and approximately 200 g of eggs in each incubator) with a constant water flow of 5 L per min and a dissolved oxygen concentration of 5.33 mg/L.

To determine the fertilization rate, 100 eggs from each female were randomly sampled and counted 8 to 12 h postfertilization (after the blastopore closure stage), and the eggs that were dividing normally were scored. Four counts were performed to determine the mean fertilization rate. At 17 h postfertilization, the overall hatching rate was determined by applying the same method described for the fertilization rate.

2.3. The GSI and morphometry of postspawning ovaries

All PGF-treated females that spawned in the three samples (4 T1, 4 T2, and 3 T3) were euthanized, after the spawn, with an overdose of anesthesia (2 g ethylaminobenzoate:150 mL alcohol:20 L water) for the collection of ovaries. In terms of controls, four control females that spawned during the first sample and four control females that spawned during the second sample were randomly chosen for the collection of the ovaries after spawning (4 C1, 4 C2). During the third collection ovaries were collected only from a single spawned female (1 C3). The gonadosomatic index (GSI) was determined with the following formula: (the total weight of oocytes released by each female + the weight of the respective remaining ovary/total body weight) × 100.

For histologic evaluation (volume density), we used ovaries from the spawned females mentioned in the previous paragraph (to determine the GSI values). We then randomly chose two C1 and two C2 (totaling four spawned controls) and two T1 and two T2 (totaling four spawned treated females [2 mL of PGF per fish]). The cranial, medial, and tail regions of the ovary tissues were fixed in Bouin solution for routine histologic procedures, embedded in Historesin for histologic preparation, and stained with hematoxylin-floxin.

2.4. Volume density

Volume density was determined using light microscopy and a 352-intersection grid. Three fields from each region of the ovary (anterior, medial, and cranial; total of nine fields) were randomly selected, giving a total of 3168 points scored for each animal at magnification X 4. For this analysis, the methodology described by de Alvarenga and de França [20] was used with certain modifications. Points were classified as one of the following: interstitial tissue (IT), previtellogenic oocyte (PV), cortical alveoli oocyte, atretic oocyte (A), immature oocytes with incomplete vitellogenesis and cytoplasm not fully filled with yolk (IM), mature vitellogenic oocytes without GVBD, and with a visible central nucleus (CNV), vitellogenic oocytes that had undergone GVBD but remained unovulated and adhered to the stroma (RV), and postovulatory follicles (POF). The percentage of each ovarian component was calculated for the four control females and four randomly selected treatment females that had received 2 mL of prostaglandin.

2.5. Statistical analysis

Normality and homoscedasticity were found for the values of fecundity, fertility, and hatching rates, which were analyzed using a Student t test ($\alpha = 5.0\%$) to compare the control and treatment groups for spawn samplings 1 and 2 during the 2009/2010 season. The frequency of ovarian cell parameters was analyzed using the nonparametric Mann–Whitney U test ($\alpha = 5.0\%$) for spawn samplings 1 and 2 during the 2009/2010 season. Statistical analysis was performed using software Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA). Data are presented as the means ± SEM. The spawning rate was used as descriptive data (without statistical analysis), and in the 2010/2011 spawning season the same counting process was performed; however, statis-

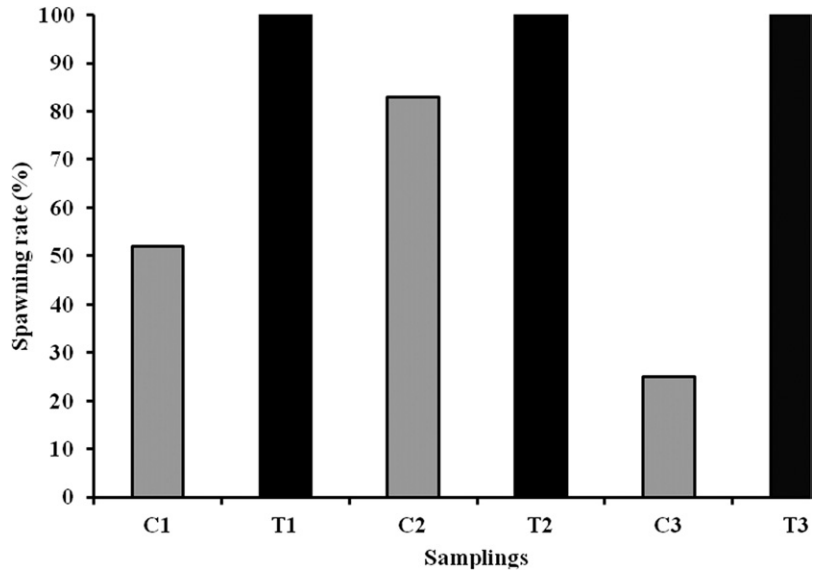

Fig. 1. The spawning rate (%) of control (C) and prostaglandin-treated (T) *P. mesopotamicus* females, as sampled during the 2009/2010 (January: T1, N = 4, and C1, N = 17; February: T2, N = 5, and C2, N = 12), and 2010/2011 (February: T3, N = 3, and C3, N = 4) spawning seasons.

tical analyses could not be performed because only one of the four control females spawned. A descriptive analysis of the oocyte volume frequency was therefore performed for this particular sampling.

3. Results

3.1. Reproductive performance

In the first and second samplings of the 2009/2010 spawning season, 100% of the females treated with PGF spawned (N = 4 in the first; N = 5 in the second), and 52.9% (N = 9) and 83.3% (N = 10) of control females spawned in the first and second samplings, respectively. In the 2010/2011 breeding season, 25.0% of the control females (one of four treated) and 100% (N = 3) of the treatment females spawned (Fig. 1).

There were no differences ($P > 0.05$) for fecundity, fertility and hatching rates of the control and treatment groups in the same sampling (Fig. 2).

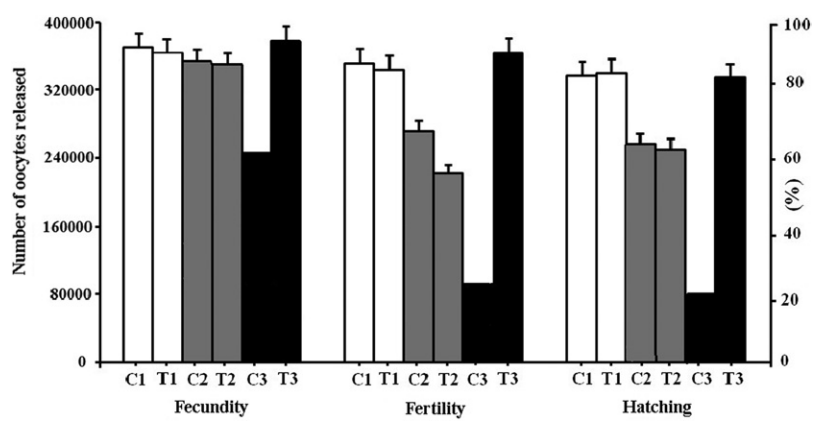

Fig. 2. The fecundity (number of oocytes released), fertility, and hatching rates of control (C) and prostaglandin-treated (T) groups sampled during the 2009/2010 (January: T1, N = 4, and C1, N = 8; February: T2, N = 5, and C2, N = 10), and 2010/2011 (February: T, N = 3, and C, N = 1) spawning seasons. The bars represent the mean values for data obtained during the 2009/2010 (January/February) and 2010/2011 (February) spawning seasons ($P < 0.05$).

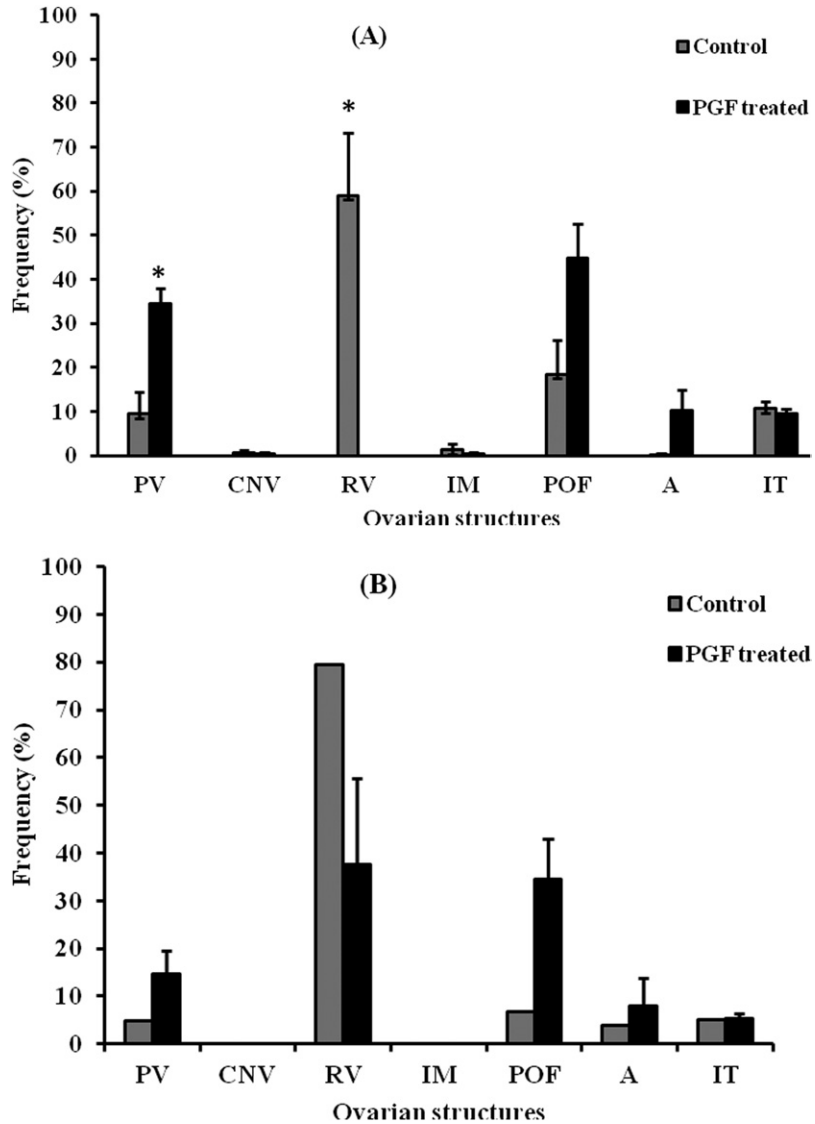

Fig. 3. The oocyte frequency (%) of the control and prostaglandin-treated *P. mesopotamicus* females as sampled during the 2009/2010 (A) (January/February) and 2010/2011 (B) (February) spawning seasons. * Significant differences ($P < 0.05$), between the frequencies of each oocyte type. A, atretic; CNV, central nucleus vitellogenic; GVBD, germinal vesicle breakdown; IM, immature; IT, interstitial tissue; POF, postovulatory follicles; PV, previtellogenic; RV, vitellogenic with GVBD but not ovulated.

3.2. Morphometry of postspawning ovaries

Morphometry was performed in sections of post-spawning ovaries with ovarian lamellae containing oocytes at various stages of development: PV, cortical alveoli oocyte, A, IM, CNV, and RV. In addition, POF and IT were also considered.

The frequency of RV oocytes was lower ($P < 0.05$) in fish treated with 2 mL of PGF ($N = 4$; $59.1\% \pm 14.1$) compared with the control group ($N = 3$; $0.0\% \pm 0.0$; Fig. 3A). The mean value for RV oocytes in fish treated with 5 mL PGF was numerically lower than the value observed in the control group (approximately 50.0% less; Fig. 3B). Treatments with 2 and 5 mL of PGF resulted in POF frequencies that were approximately two and three times higher, respectively, than the frequencies in the control groups, but the observed differences were not significant ($P > 0.05$). In fish treated with 2 mL of PGF, the frequency of PV remaining in the ovaries after spawning was higher than in the control group ($P < 0.05$; Fig. 3A). A similar profile was observed in fish treated with 5 mL of PGF (Fig. 3B). The frequency of CNV, IM, A, and IT did

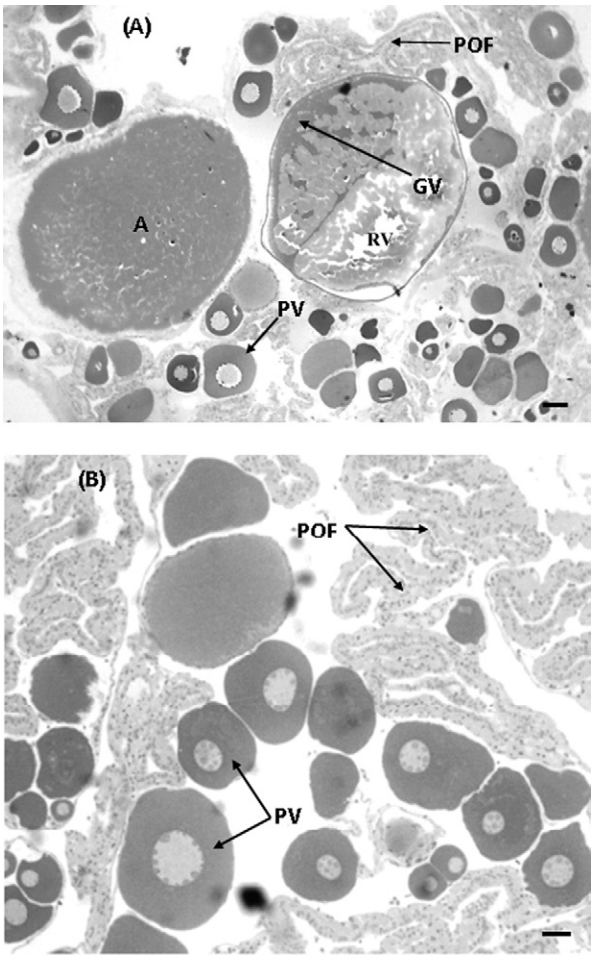

Fig. 4. The photomicrography of the control (A) and 2 mL prostaglandin-treated (B) *P. mesopotamicus* ovaries. The postovulatory follicles (POF), vitellogenic with germinal vesicle breakdown (GVBD) but unovulated (RV), previtellogenic (PV), atretic (A) and germinal vesicle (GV). Observe that in an RV oocyte, the germinal vesicle breaks down and migrates to the periphery of the cell, completing final maturation, but ovulation has not occurred. Hematoxylin-floxin. Bar = 10 μM.

not differ between the control and treatment groups ($P > 0.05$; Fig. 3A). The morphologic aspects of the remaining ovaries of the control and treated females are shown in Fig. 4.

4. Discussion

The use of PGF (Ciosin/Schering-Plough) effectively improved the reproductive performance of pacu. The spawning rate in the treated animals was constant for all three spawn samplings (100%), whereas the spawning rate for the control group varied widely, ranging from 25.0% to 84.0%. In addition, morphometric analysis revealed that PGF specifically affected the ovulatory process, intensifying it in treated females.

In the present study, exogenous PGF significantly reduced the number of oocytes remaining in the ovaries, which corresponded to the number of oocytes that had undergone GVBD (final maturation) but remained unovulated. This finding corroborated previous work that a higher percentage of GVBD oocytes in the ovaries of *Brycon amazonicus* females that did not spawn [6]. Thus, a failure in the ovulatory process is clearly linked to the absence of spawning in tropical migratory total spawner species.

The role of prostaglandins in the ovulatory process of tropical total spawner migratory species, which represent the majority of commercially relevant fish in Brazil, remains unknown. Most studies have used fish models or in vitro approaches in species from temperate regions for which a direct association between ovulation and prostaglandins has already been demonstrated. Prostaglandin induced GVBD and ovulation in yellow perch *Perca flavescens* [21] and in *Salvelinus fontinalis* [22]. Similar results were obtained in vitro in *Brachydanio rerio*, in which the inhibition of prostaglandin concentrations by the use of indomethacin, an inhibitor of the cyclooxygenase enzyme that acts on prostaglandin synthesis, caused a reduction in the number of ovulated oocytes [13]. In *P. mesopotamicus*, a reduction in the number of GVBD oocytes retained in the postspawning ovaries of treated females was observed, suggesting that the role of PGF is related to the process of ovulation. Future studies utilizing in vitro approaches may address whether this substance is also related to the induction of final maturation and GVBD.

It remains unclear why some pacu control females that were hormonally induced with pituitary extract spawned, whereas others did not. One possibility is that females that failed to spawn had inadequate concentrations of PGF, which could help explain the effects observed when this substance was administered exogenously. New approaches are needed to assess whether there is a possible correlation between increased PGF concentrations and successful ovulation. It is well established that prostaglandins are naturally produced and secreted by females of several fish species during spawning [23]. Lister and Van Der Kraak [14] demonstrated that in vitro follicles synthesize PGE_2 and $PGF_{2\alpha}$ in the presence of arachidonic acid in a dose-dependent fashion. Several other studies have also shown a relationship between successful ovulation after hormonal induction and increased prostaglandin concentrations [9–12].

In the present study, there was no apparent dose-dependent relationship for PGF. When 5 mL was used (third sampling), only one control female spawned; as a result, it was not possible to statistically analyze the spawning and histomorphometric data. Based on the observed spawning rate and other reproductive parameters evaluated in this study, there was no evidence for a dose-dependent effect.

The use of PGF did not affect the rates of fertility (an average of 77.6% from three spawn samplings) and hatching (an average of 69.7% from three spawn samplings) when treatment and control fish from the same sampling were compared. Similarly, Guerreiro et al. [24] reported values of 67.0% and 51.0% for fertility and hatching rates, respectively, indicating that PGF is a safe substance for the induction of spawning. Presently, there are no scientific data demonstrating that the use of this hormone reduces the fertility and hatching rates of fish or causes malformations of larvae.

The findings of the present study may address some of the limitations associated with induced spawning in pacu and other tropical migratory fish. Unsuccessful or unpredictable spawning of females after hormone treatments has tremendous financial consequences, especially in the case of *P. mesopotamicus*. In the present study, the use of exogenous PGF led to 100% spawning rates in treated females and a significant decrease in the number of oocytes with GVBD retained in the ovaries postspawning. The use of this substance did not affect the fertility and hatching rates, and no malformed larvae were observed. Novel approaches are needed to address the precise role of prostaglandins in ovulation and to determine whether successful spawning in pacu is correlated with PGF concentrations before and after spawning. Moreover, the most effective doses and periods of administration should be established in future studies.

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